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EFFECTS OF PROCESSED SWEET POTATO DIETS ON THE KINETICS OF SPERMATOGENESIS IN BROILER BREEDER COCKS

(*Gallusgallus domesticus*)

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ABSTRACT

There is growing need to replace maize in poultry feeds with tropical root and tubers, hence the need to establish the effect of such feeds on the animal's reproductive potentials. Although the male's sperm-producing capacity is genetically-determined, several other external factors (photoperiod, nutrition, humidity, stress, and toxic substances) may contribute to determine what portion of the germ cells are actually delivered for reproductive purposes. The kinetics of spermatogenesis, volumetric proportion of testicular elements and diameter of seminiferous tubules were studied in 20 adult cocks (Yaffa-breed) fed processed sweet potato diets for ten weeks. Data were analyzed using randomized complete block experimental design. The results showed significant treatment effects (P< 0.05) between the control and the sweet potato diets on the volumetric proportion (%) of the testicular elements. Spermatogonia appeared highest (3.73 ± 0.08) in the grated sweet potato (GP_{25}) and least (3.15 ± 0.12) in the control maize-based diet. Spermatocytes equally appeared highest (10.80 ± 0.28) in GP_{25} and least (9.25 ± 0.12) in thinly sliced sweet potato (SP_{25}). There was no significant treatment effect in the spermatids, while spermatozoa were highest (4.40 ± 0.08) in the control diet and least (2.44 ± 0.12) in SP_{25}. Sertoli cells ranked highest (2.13 ± 0.08) in the control diet and least (1.05 ± 0.20) in SP_{25}. Leydig cells showed no significant treatment effect. Processed sweet potato diets favoured spermatogenesis with resultant potentially fertile cocks. Sweet potato is therefore an economically viable substitute for maize with no effect on the animal's reproductive potential.

Novelty Statement

Brought out the effect(s) of sweet potato meal on the reproductive potential of the cock

Proven that sweet potato can be a potential component in the feed of domestic fowls

Shown that sweet potato has no detrimental effect(s) on the spermatogenesis of the cock.

Shown that sweet potato is a palatable substitute for maize (which is in very high demand for other purposes) in the feed of the cock.

KEYWORDS: Poultry Feed, Spermatogenesis, Breeder Cocks, Sweet Potato, Reproduction

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Original Article

INTRODUCTION

In a bid to improve livestock in the sub-Sahara Africa, tropical tubers have been used to substitute maize in feeds. So far, emphasis has been on the animal's performance in terms of feed intake and weight gain. It is but obvious that animal production is a function of animal reproduction. Beside genetic factors, the reproductive

potential of an animal is influenced by the other external or environmental factors. It is important to note that the total sperm producing capacity of the male animal is determined genetically but there are many factors which determine what portion of the germ cells are actually delivered for the reproductive purposes (Hamner, 1970). The important external or environmental factors affecting sperm production and development include temperature, photoperiod humidity nutrition stress and toxic substances.

Frandson (1986) defines spermatogenesis as the process by which primary sex cells in the testis produce spermatozoa. Spermatogenesis is a development process which involves several stages and different cells sequentially leading to one another to finally release the mature cells called spermatozoa. The Seminiferous tubules of the immature males are lined by a single-cell layer of sertoli cells and spermatogonia while mature males have irregularly shaped tubules lined by a multi-layered germinal epithelium (Harper, 1987). The spermatogonia are involved in mitotic cell division to produce the generation of germ cells called Spermatocytes (Clermont, 1972; Roosen-Runge, 1977; Steinberger and Steinberger, 1975). Spermatogonia are involved in mitosis because they do not have mature DNA for meiosis. From spermatogonia A and B is considered the stage when almost all the dna is formed for meiotic divisions (Gichereau, 1967).

The amount of day light has an effect on the pituitary gland. Sperm production deceases in hot climates probably due to the direct effect of heat on the testes (Frandson, 1986). However, Nkanga(1989) and Egbunike and Nkanga (1999) reportedsemen volume and sperm concentration to be significantly favoured in the early dry season (October to December) than early rainy season (April to June). Low environmental temperature do not seem to affect semen production (Hamner, 1970)

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With scanty literature available on nutritional influence on spermatogenesis, it is therefore necessary that this study should be carried out especially with the need to substitute maize with tropical tubers to curb cost of livestock production. The objective of this study was therefore to assess the effect of processed sweet potato-based diet on spermatogenesis in breeder cocks in the early rainy season through evaluation of their

- Kinetics of spermatogenesis
- Volumetric proportion of testicular elements
- Diameter of the Seminiferous tubules

MATERIALS AND METHODS

Paired Testes Weight/Epididymal Weight

The left and right testes of each cock for each treatment were measured separately and their weights were recorded to the nearest 0.01g. The left and right epididymides weight were also measured separately to the nearest 0.01g.

Testes Volumes

The volumes of the testes were determined by Archimedes' principle of water displacement and the result recorded in milliliters.

Testes Density

The testes density was calculated from the testis weight and volume and expressed in g/cc.

Tunica Albugineal Weight

The tunica albuginea was carefully removed from the testis starting from the doral surface. Thiswas then weighed and the weight recorded to the nearest 0.01g.

Testicular Histology

Fixation: Samples from the right testis of the treatment were fixed in about ten times their volume in aqueous Bouin's fixative for 24 hours. Bouin's fixative is composed of 75ml of picric-acid, 20ml of formalin and 5ml of glacial acetic acid.

Dehydration and Embedding

Following fixation, the tissue samples were left in 70% ethyl alcohol (ethanol EtOH) and thereafter dehydrated in increasing concentrations of ethanol and cleared in three changes of chloroform as follows

$$70\%\ EtOH \longrightarrow 80\%EtOH \longrightarrow 95EtOH \longrightarrow Paraffin \longrightarrow CHCL_3$$

$$CHOL_3 \longrightarrow CHOL_3 \longrightarrow 100\%\ EtOH \longrightarrow 100\%EtOH$$

Each sample was allowed one hour in each alcohol and chloroform change and was left overnight in paraffin (Wax) at 60%. The cleared tissue samples were impregnated under pressure for 30minutes and embedded in wax to form tissue blocks. Histological sections of 7 microns thick were floated and flattened out on 40^{o}C water, and then picked up carefully with clean slides smeared with Mayer's egg albumin. These slides were stored in an incubator for 30minutes before staining

Stain and Staining Procedure

Two sets of slides were prepared for each animal. One set was stained according to the periodic-Acid-Schiff (PAS) technique and counter stained with haematoxylin. The second set was stained with haematoxylin-eosin (H&E).

Spermatogenesis

The stages of transformation of the round spermatid to a free spermatozoon were identified by a combined method of assessment of acrosome development as revealed by PAS technique (Leblond and Clermont, 1952a) and nuclear morphology revealed by haematoxylin-eosin stained paraffin embedded tissue (De Reviers, 1971).

Identification of Spermatogenic Elements and Cellular Associations

Cell repeatedly associated together at the different stages of spermatogenesis were identified with respect to their various reactions following the staining procedures. Spermatogonia were identified by their position next to the basement membrane and by their nuclear diameter as well as the distribution of marginal heterochromatin and the chromatin proper. The Spermatocytes were identified by their nuclear diameter, their position in the seminiferous epithelium and the meiotic activity of their chromatin. The spermatids were identified by the alteration of the shape and position of their nuclei following elongation as well as the development status of the acrosomic system of young spermatids.

Meanwhile Sertoli cells were seen closely adjacent to each other between which the germ cells which develop into spermatogonia are found. Most of the identification from spermatogonia to spermatids is based on the size of the nucleus and chromatin mineral.

Volumetric Proportions of Spermatogenic Elements

The volumetric proportions of spermatogenic elements in the seminiferous epithelium were determined using the methods of Chalky (1943). A twenty five point ocular graticule (Carl Zeiss, Oberkochen) was used in the estimation. Microscopic fields were observed at random and the structure, including artifacts, under each 'hit' was recorded. Precautions were taken to ensure that no field was examined twice. Forty fields were observed for each slide. All observations were made on the 7-microns sections using a x10 ocular and a x100 oil immersion objective. The testicular elements classified included

i) Spermatogonia type A**ii)** Round spermatids**iii)** Basement membrane**iv)** Cellular cytoplasm**v)** Spermatogonia type B**vi)** Elongated spermatids**vii)** Leydig Cells**viii)** Lumen

ix) Primary Spermatocytes **x)** Free Spermatozoa**xi)** Interstitial cells other

xii) Intertubular cell space than those of Leydigspace

xiii) Primary Spermatocytes**xiv)** Sertoli cells**xv)** Interstitial cells spaces

Statistical Analysis

All data collected were subjected to analyses of variance using SAS (1999). The means were separated using Duncan's multiple range tests

RESULTS

Spermatogeneses

Four basic cell types in addition to few others were recognized among the geminal cell of all cocks. Apart from the spermatozoa these cells include the spermatogonia, spermatocytes and spermatids. The semiferous epithelium of the cock was composed of in addition to sertoli cells two generations of spermatogonia, two forms of spermatocytes and two generations of spermatids. Sertoli cells were observed to be the only non-geminal cells in the semiferous epithelium. The basement membrane of semiferous tubules was lined by spermatogonia of two types, A and B, which could be identified on the basis of their nucleus and chromatin material. Plasma membrane from tight junctions between adjacent Sertoli cells divides the semiferous epithelium into compartments. These compartments constitute the sides for mitotic and meiotic divisions. The basal compartment consists of the germ cells dividing mitotically until they become committed to meiosis.

These comprised of the spermatogonia and the preleptotene spermatocytes. In the other compartment close to the lumen otherwise called the adluminal compartment, germ cells in meiotic prophase complete meiosis to produce secondary spermatocytes. The spermatids resulting from the secondary maturation division of the secondary spermatocytes also complete spermiogenesis (process by which round spermatids is transformed into spermatozoa by a series of progressive morphological changes) in this compartment.

In a nutshell, the various cells can summarily be described as below. This description is done to ease the eventual identification of the cellular associations and the stages of the cycle of the semiferous epithelium.

Spermatogonia Type A

These cells were found lining the laminal of the seminiferous tubule. Spermatogonial type A are characterized by a large pale ovoid nucleus ranging from 5.75 to 7.90 microns in diameter. They also possess a nucleolus centrally or acentrally located with fine homogeneous chromatin granules which are dust-like in appearance.

Spermatogonia Type B

The B-type spermatogonia are a result of the mitotic division of spermatogonia type A. Like A-type, B-type spermatogonia are diploid. They are characterized by a dark nucleus which is smaller, 4.25-6.70 microns, in diameter and more nearly spherical in shape. Its chromatin material appeared crust-like and even more pronounced i.e. more visible than those of spermatogonia type A.

Sertoli Cells

These cells were seen to support the spermatogenic epithelium. They were in a way traced from the basement membrane of the semiferous tubules to the adluminal compactment where the spermatozoa were released into the lumen. They were easily identified by their characteristic irregular shapes with deep invaginations of nuclear membrane. Their chromatins were seen to form a sparse network in the nucleoplasm which becomes dense around the nuclear membrane. They have a prominent nucleus which was more or less centrally located. The Sertoli cell nucleus was separated from the basal lamina by an almost continuous layer of spermatogonia. The size of the sertoli cell nucleus varies depending on the stages of the cycle of semiferous epithelium.

Primary Spermatocytes

They are formed by the last spermatogonia mitosis of spermatogonia type B.Morphologically, they resemble the **B-** spermatogonia. The only difference is that their crust of chromatin in the nucleus has filaments within them which were really distinguishable. They had more visible nucleoli and chromosomes. Paired chromosomes were seen to be intensely stained and appeared to be attached by their extremity to a given area of the nucleoplasm to form a characteristic crescent shape with the nucleus (Zygotene phase). The nucleus at this point ranges from 5.95-7.00 microns in diameter. The thickening and condensation of chromosomes intiated at zygotene continued with a corresponding increase in the nuclear diameter and volume. This was the pachytene primary spermatocyte. By this time, the nuclear measured 6.75-7.50 microns. It was later observed that the chromosomes condensation was at its maximum such that they appeared as intensely stained granules in the nucleoplasm. The nucleus has alsoattained its maximum size 7.55-7.95 microns. This was the diplotene primary spermatocyte. After diplotene, a brief period of division (diakinesis, metaphase, anaphase and telophase) followed resulting in the formation of secondary spermatocytes.

Secondary Spermatocytes

They had spherical nuclei with a network of chromatin granules like the type observed in the first phase of spermatid development, but larger than the round spermatids (5.00 - 7.50 microns) and were observed only at stage VIII of the cycle of seminiferous epithelium.

Spermatids and Spermatogenesis

These cells resulted from the second meiotic division. Initially, they appeared as small round cells with a pale nucleus and a moderate amount of undistinguished cytoplasm. They start with the appearance of round spermatids and end with the release of spermatozoa in the lumen. That is, from the round spermatids to elongated spermatids, these cells were then transformed into the very complex spermatozoa, undergoing condensation of the nucleus,formation of the acrosome, virtual elimination of the cytoplasm, development of the tail and the arrangement of its mitochondia into a helix to produce a midpiece. This process is known as spermatogenesis or spermateleosis

Volumetric Proportions of Testicular Elements

The results of the volumetric proportions of testicular Elements in breeder cocks are presented in table 1. significant difference ($P<0.05$) were observed in most of the testicular elements while some of the testicular elements (like the secondary spermatcytes, round spermatids, elongated spermatids, basement membrane, interstitial cell, interstitial cell space and Leydig cell) showed no significant treatment effect. Amongst those testicular elements that showed significant treatment effect spermatogonia types A and B were higher in the sweet potato-based diets than the control. Treatment GP_{25} recorded the highest (3.73 ± 0.08%) and (2.21 ± 0.18%) proportions of spermatogonia A and B respectively. The control diet had the least (3.15 ± 0.12% and 1.60 ± 0.08%) proportions of spermatogonia A & B, respectively. Treatment GP_{25} still recorded the highest (10.80 ± 0.28%) proportion of primary spermatocyteswhile treatment SP_{25} had least (9.25 ± 0.12%)proportions of primary spermatocytes. Theproportions of spermatozoa for the different dietary treatment were 4.40± 0.08, 4.05 ± 0.12, 2.53 ± 0.23 and 2.44 ± 0.12 for the control$FP_{25}GP_{25}$and SP_{25}, respectively. Treatment SP_{25} tended to have the highest (51.33 ± 0.08%) in the proportion occupied by the cytoplasm while the control diet had the least and (50.20 ± 0.43). The mean proportion of seminiferous tubules in the testes was highest(89.15 ± 0.38%) in treatmentGP_{25} and least (86.36± 0.20%) in the diet SP_{25}

Table 1: Volumetric Proportion (%) of Testicular Elements in Breeder Cocks Feed selected Sweet-Potato-Based Diets in the Early Raining Season

Sweet Potato Treaments				
Elements	**Control**	**GP_{25}**	**FP_{25}**	**SP_{25}**
Spermatogonia A	3.15 ± 0.13^{b}	3.73 ± 0.08^{a}	3.25 ± 0.12^{b}	3.67 ± 0.04^{a}
Spermatogonia B	1.60 ± 0.08^{b}	2.21 ± 0.18^{a}	1.87 ± 0.15^{b}	1.96 ± 0.24^{ab}
Primary spermatocytes	9.38 ± 0.77^{ab}	10.80 ± 0.28^{a}	10.40 ± 0.16^{a6}	9.25 ± 0.12^{b}
Secondary spermatocytes	0.23 ± 0.02	0.31 ± 0.12	0.36 ± 0.16	0.27 ± 0.04
Round spermatids	4.64 ± 0.67	4.53 ± 0.38	4.67 ± 0.15	4.76 ± 0.16
Elongated spermatids	8.01 ± 0.97	8.44 ± 0.12	8.13 ± 0.15	8.10 ± 0.19
Spermatozoa	4.40 ± 0.08^{a}	2.53 ± 0.23^{b}	4.05 ± 0.12^{a}	2.44 ± 0.12^{b}
Sertoli cells	2.13 ± 0.08^{a}	1.07 ± 0.15^{b}	1.89 ± 0.13^{a}	1.05 ± 0.20^{b}
Basement membrane	2.80 ± 0.08	2.66 ± 0.14	2.62 ± 0.09	2.49 ± 0.16
Lumen	3.61 ± 0.08^{a}	2.36 ± 0.16^{b}	3.47 ± 0.15^{a}	2.36 ± 0.28^{b}
Interstitial cells	0.97 ± 0.05	0.87 ± 0.10	0.93 ± 0.15	0.98 ± 0.12
Interstitial cells space	0.64 ± 0.06	0.49 ± 0.10	0.60 ± 0.04	0.62 ± 0.16
Cytoplasm	50.20 ± 0.43^{b}	50.55 ± 0.29^{ab}	50.67 ± 0.15^{ab}	51.33 ± 0.08^{a}
Leydig cells	0.97 ± 0.05^{a}	1.11 ± 0.16	1.07 ± 0.15	0.98 ± 0.03
Intertubular space	3.40 ± 0.04^{a}	2.63 ± 0.09^{b}	3.45 ± 0.20^{a}	3.19 ± 0.08^{a}
Seminferous tubules	86.67 ± 0.15^{bc}	89.15 ± 0.38^{a}	87.12 ± 0.20^{b}	86.36 ± 0.20^{c}

abc: Treatment means with different superscripts along the same row are significantly different($P<0.05$)

Diameter of Seminiferous Tubules

The mean tubular diameter is shown in table 2. There was a significant difference ($P<0.05$)between the dietary treatments.

Table 2: Effect ofSweet-Potato-Based Diets on the Diameter of Seminiferous Tubules of breeder cocks

Parameter Sweet potato Treament				
	Control	**GP_{25}**	**FP_{25}**	**SP_{25}**
Diameter (u)	249.45 ± 1.47^{a}	200.56 ± 3.38^{b}	272.78 ± 1.83	215.01 ± 2.66^{b}

ab: Treatment means with different superscripts along the same row are significantly different ($P<0.05$)

DISCUSSIONS

Spermatogenesis

Spermatogonia

Two categories of spermatogonia (A&B) were described here based on the sizes of their nuclei, chromatin materials and position in the tubule. These results are similar to earlier descriptions of these two spermatogonia by De Reviers (1971), Nkanga and Egbunike, (1990) and Cerolini et al (2003). Miller (1938) described only one type of spermatogonia without outlining the criteria used for identification. Lake (1956) and Surai (1992) respectively described two categories of spermatogonia. From their description type-two spermatogonia in this particular study is actually primary spermatocyte undergoing the first maturation division (diplotene dividing to produce secondary spermatocytes), and their type 1 spermatogonia corresponds to primary spermatocytes at zygotene. The dormant type spermatogonia, which they descrined are actually the B-type spermatogonia. These authors used the phrase dormant type spermatogonia due to their knowledge about DNA formation as at that time. Presently, the phrase dormant type spermatogoniahave fallen out of use due to advance knowledge in nucleic acid synthesis. The spermatocytes formed by the last spermatogonial mitosis are now called “preleptotene spermatocytes” that used to be called “resting or dormant spermatocytes” by morphologists, but since it has been shown that this is the stage when all the DNA is formed for the meiotic divisions, this term has rightly fallen out

of use. Morphologically these cells resemble the B type spermtogonia. Furthermore, three categories of spermatogonia, A, in and B each with an ovoid nucleus were identified for the Japanese Quail (Yamamoto, et al. 1967). Nevertheless, the intermediate type (In) spermatogonia could not be demonstrated in the domestic fowl. Indentification of In spermatogonia in the domestic fowl, was based on the nuclei size, chromatin configuration and relative position of these cells in the seminiferous tubules. The results of this study are similar to those of the last three authors in that beside the same method of identificationadopted, two categories of spermatogonia were identified in the breeder cocks used. Also the dietary treatment used did not influence the identification and the spermatogonia identified were not significantly influenced by the dietary treatments.

Primary Spermatocytes

Five categories of primary spermatocytes were recognized in tubular cross-section of the breeder cocks used. These include the preleptotene, leptotene, zygotene, pachytene and diplotene. The spermatocytes formed by the spermatogonial mitosis are now called preleptotene spermatocytes. Morphologically these cells resemble the B spermatogonia but when filaments become distinguishable within the crusts the chromatin in the nucleus, these cell have entered leptotene,the real beginning of the meiotic prophase. By the end of this stage, spiralization and contractions of the chromosomes occur just after DNA sythesis has stoppes. Therefore at this time, these cells contain a tetra-ploid amount of DNA. During the next stage, zygotene, the analogous chromosomes pair off and become thicker, then gather in a bouquet-like arrangement. Synaptinemal complexes appear between pairs of chromosomes and the nucleoli become more easily visible. The next stage is the pachytene which is considered the longest in the meiotic prophase. Hence each chromosomes thickens and begins to show longitudinal split, except at the level of the centromeres. Finally, in diplotene the nucleus reaches its maximum size and duplication of the chromosomes is complete so that complete tetrads are formed. The next stag diakinesis is a very rapid one and is the stage when separation occurs though it was not observed. It can be seen in rodents(Clermont and Leblond, 1953) but extremely rapid and therefore sheldomly observed in rams and bulls (Clermont, 1954). The members of homologous chromosomes separate into two secondary spermatocytes.

Pachytene primary spermatocytes were the most freguent encountered type in all the stages of the seminiferous epithelial cycle except stage VII where they had become differentiated into diplotene. Zygotene steps occurred in stages V-VII while the Leptotene were observed in stages III and IV. Pre-lephtotene primary spermatocytes were observed in stages I and II as a result of mitotic division of the B-type spermatogonia at stage VIII. Five categories of primaryspermatocytes were reportedby De Reviers (1971), Nkanga and Egbunike, (1990) in the cock while Aire et al (1980) reported three classes of leptotene, zygotene, pachytene in the gunea fowl. The difference in number of categories and classes of primary spermatocytes identified by the varios groups of authors may be due to differences in species of poultry used. No significant treatment effects were observed and as such the five categories of cells were identical in all the four dietary treatments.

Spermatids and Spermiogenesis

The second maturation or meiotic division gives rise to spermatids. The maturing spermatids undergo about 10cellular stages befor they are released from Sertoli cells. This is based from the observations made on haematoxhlin-eosin section in which 10 stages of spermatid development were identified. The end product of these 10stages is that the cells are finally transformed into the very complex spermatozoa which then undergo some processes known as spermiogenesis or spermateleosis. It is a complex process. As the spermatids develop in close association with the Sertoli

cells the latter may well be actively involved. Ganner and Hafez (1993) using the Periodic Acid-Schiff (PAS) could identify four developmental stages of spermatid transformation to spermazoa. These include the Golgi, cap, acrosormal and maturation phases. Cavazos and Mellampy (1954) and De Reviers (1971) with H and E sections recognized 15 and 8 stages respectively in the domestic fowl, while Gunawardana (1977) with araldite sections reported 10 stages of development. Also Nkanga (1989) and Nkanga and Egbunike, (1990) recognized 10stages in the domestic fowl. In this particular study 10

Stages were also recognizes. However, Gunawardana (1977) description of stage 4 actually corresponds to stage 5 of the present study since the nucleus was already pear shaped. The stage 4 spermatid is almost a round cell with some evidence of elongation. At this stage, the acrosomic granule had collapsed on the nuclear membrane giving the cell a more or less crescent shape with intensely staining periphery and pale staining nucleoplasm. Similar observations were made by and De Reviers (1971); Nkanga (1989) and Maldjian et al. (2002). The reports of Cavazos and Mellampy (1954) agree with those of Ganner and Hafez (1993) in which they both reported the presence of a cap phase during spermatid differentiation in the domestic fowl. The authors observed the same cells but the problem which was some how particular was that of ascribing a definite description to the particular cell (s) identified. This partly accounted for the authors either recognizing the presence or absence of the cap phase. In this investigation, the stage 4 spermatid had a cap-like appearance suggesting the presence of a cap phase. De Reviers (1971) observed this stage but described it as crescent or star-shaped.

The Golgi phase was recognized to be represented by stages 1,2 and 3. the dark staining nuclear periphery and the pale staining cytoplasm of stage 3 is recognized as the beginning of nuclear condensation had been reported by De Reviers (1971) and Gunawardana (1977) as was the acrosome granule. Stages 5, 6,7and 8 were identified by their nuclear morphology, the acrosome structure and relative length.

The changes in nuclear morphology are accompanied by the displacement of the cytoplasm to the caudal aspect of the nucleus, where cytoplasm surrounds the proximal portion of the developing tail. Here the nuclear constriction observed in stage 6 smoothens out into grentle waves while increasing spermatid length was observed in subsequent stages. Coiling of the nucleus of elongating spermatids was reported by Zlothik (1947) and De Reviers (1971) who however, considered the coiled forms abnormal. From the description of spermatid provided by De Reviers (1971) it appears that stages 4, 5, 6 and 7 of this investigation were represented by her stages 4 and 5 while stage 8 corresponded to her stage 6. This observation was also reported by Nkanga and Egbunike, (1990). Also this same observation was made by Gunawardana (1977). Meanwhile the identification of stages 9and 10 was based on the relative position of the maturing spermatid to the basement membrane and their staining characteristics. Thus stage spermatid was found at the border of the lumen with the nucleus intensely stained and bent with a curvature.At stage 10 the spermatid was more intensely stained, was more intensely stained, was more at the border of the lumen and had matured into a double curved spermatozoon. These stages 9 and 10 correspond to the maturation of phases observed and described by Ganner and Hafez (1993).

Volumetric Proportion of Testicular Elements

Significant differences ($P<0.05$) was observed in some of these elements (Spermatogonia types A and B, Primary spermatpcytes, spermatozoa Sertoli cells, lumen, cytoplasm, intertubular space and seminiferous tubules) while some of these elements (Secondary spermatocytes, both round and elongated spermatids, basement membrane, interstitial cell space and Leydig cells) had no significant treatment effect. These results can be appreciated with adequate explanation from knowledge of the kinetics of spermatogenesis and cytology. Spermatogonia A ranged from the highest value in treatment

GP_{25} (3.73 ± 0.08%) to the least value in the control diet (3.15 ± 0.12%). Likewise spermatogonia B were higest in GP_{25} (2.21 ± 0.18%) and least in control diet (1.60 ± 0.08%). Spermatogonia A and B are both involved in mitotic cell division in the whole process of spermatogenesis with a diploid number of chromosomes. Though these results agrees with those of De Reviers (1971) and Nkanga and Egbunike (1990). The further that diets GP_{25}andSP_{25}encouraged mitotic cell division than $FP_{25}$and the control diet. Up to the level of production of primary spermatocytes, diet GT_{25} still had the highest proportion of primary spermatocytes (10.80 ± 0.28%) compared to the least value (9.25 ± 0.12%) obtained in dietSP_{25}. Fermented sweet potato(FP_{25}) had its sugar content reduced to the bearest minimum duringthe three days of fermentation,while thinly sliced sweet potato (GP_{25}) had just moderate concentrations of sugars in its final product. These variations in the proportion of soluble sugars influence spermatogenesis at the level of mitosis in which spermatogonia A and B are solely involved. Poultry is sensitive to its diets and the slightest imbalance to composition goes a long way to influencing its physiology and subsequently the performance. Cock semen is low in fructose (4mg/100ml) and 7.7 to 81mg/100ml of glucose. The glucose is thought to have originated from the cloacal glands rather than from ejaculatory ducts (lake, 1966; Hamner, 1970). Meanwhile Etchu and Egbunike (2002) reported the total soluble sugar in various forms of processed sweet potato to be 4.90% fermentation in water for three days, 9.85% for thinly slice and 10.24% for grated sweet potato. From the report of lake, (1966), Hamner, (1970) and Etchu and Egbunike (2002), the results of this present investigation were therefore obvious that any feed type that would add to the level of essential soluble sugars in the cock semen, will encourage the production of germ cells and subsequently spermatogonia. Every species has its capacity for sperm production that is genetically determined, but other external factors (like nutrition disease, stress etc) influence which portion of the germinal epithelium to enter into spermatogenesis (Garner and Hafez, 1999). This further explains why the proportions of primary spermatocytes were highest in diet (GP_{25}). However, following the production of secondary spermatocytes from the first meiotic division of primaryspermatocytes, and the other products (round and elongated spermatids)from the second meiotic division, no significant treatment effect was observed. These results agree with earlier reports by Amann (1981), Nkanga, Nkanga (1989), and Egbunike (1990). The latter authors worked with two breeds of domestic of domestic fowl. However, it is worthy to note that secondary spermatocytes, round, and elongated spermatids as well as the spermatozoa contain haploid number of chromosomes. This implies that irrespective of the form of processing or diet type sweet potato diets have little or no influence on meiotic cell division of spermatogenesis. Diakinesis itself was a very transient stage observed only in stage VIII, sometimes with secondary spermatocytes in the second maturation division. In addition to this, each stage of the cycle of the seminiferous epithelium had at least one form of spermatid, either round or elongated. With this dispersed and relatively even distribution, all the dietary treatment had a numerical difference in these haploid cells which were not statistically significant.

A high correlation was observed between spermatozoa and Sertoli cells. Dietary treatments that had higher proportions of spermatozoa were also observed to have a corresponding higher proportion of Sertoli cells. These finding agree with those ofDe Reviers (1971), Amann (1981),and Nkanga and Egbunike (1990). The high observed correlation between spermatozoa and Sertoli cells can be explained by the fact that the morphological transformation of spermatids during spermatogenesis occurs with the spermatids embedded within cytoplasmic pockets of individual Sertoli cells. In addition to this, Sertoli cells which support the spermatogenic epithelim, have endocrine activity and participate in spermiogenesis (McDonald, 1981), by phagocytosis of the residual bodies shed by the maturing spermatids. The release of spermatozoa from the cytoplasmic pockets of the Sertoli cells. Spermination, involves marked swelling of the Sertoli cells(Gresson and Zlotnik, 1948 and McDonald, 1981).

The dietary treatment did not influence the proportion of the basement membrane. This is partly due to the fact that the vascularsupply to the germinal epithelium is outside the basement membrane of the seminiferous tubule(Gresson and Zlotnik, 1948). With this type of vascular arrangement, the diet will exert little or no influence on the proportion of basement membrane in the seminiferous tubule. The lumen was also a reflection of the spermatozoa and Sertoli cells. This is partly because once the proportion of spermatozoa and Sertoli cells is high, there must be a corresponding increase in the proportion and size of the lumen to accommodate the released spermatozoa.

The volume percent occupied by the seminferous tubules also showed significant treatment effect. There was a correlation between the proportion of seminiferous tubule and spermatogonia. Treatment $GP_{25}$with the highest volumetric proportion of spermatogia types A and B and also had a correspondingly higher proportion of seminiferous tubule (89.15± 0.38%) though this trend did not hold for all the other diets. However, from these results coupled with earlier reports by Garner and Hafez, (1999) one can categorically state that treatment GP_{25} encouraged a larger proportion of the germinal epithelium of the seminiferous tubule to enter into spermatogenesis. This explains why treatment GP_{25} had the highest volumetric proportions of both spermatogonia A and B, primary spermatocytes as well as the seminiferous tubule. What would have influenced these results is partly due to the course nature of grated sweet potato based diet (GP_{25}) which the cock could consume maximally for efficient production compared to the other forms of sweet potato-based diets. Moreover, effective performance and reproductive potential is a function of how efficient the animal consumes and makes use of the nutrients in the feed. This result on the proportionof seminiferous tubules agrees with those of Nkanga and Egbunike (1990). The figures obtained here are slightly lower than those reported for adult cocks of 32 weeks by De Reviers (1971); The variation in volume percent reported for domestic fowl (De Reviers, 1984), dairy bulls (Amann, 1962), boars (Kennelly and Foote, 1964), ram (Bascom and Osterud 1956), rat (Roosen-Runge, 1956) and Man (Bascom and Osterud 1956); Roosen-Runge, 1956 seems to indicate that the volume percent of seminiferous tubules in the testis is species specific.

Diameter of Seminiferous Tubules

Tubular diameter range from the highest (272.78± 1.83micros) in treatment FP_{25} to the least (200.56± 3.38micros) in treatment $GP_{25.}$ The control diet and diet FP_{25} had a numerical difference in tubular diameter which was not statistically significant. Likewise diet GP_{25} and SP_{25}had a numerically difference in tubular diameter which was not statistically significant. These values of tubular diameter for the respective dietary treatments are correlated to their proportion of Sertoli cells in tubule. This correlation is partly because the Sertoli cells serve to convey nutrients and metabolites between the spermatogenic cells and the peritubular capillaries (McDonald, 1981). In line with their role in substaining maturing germinal elements during spermatogenesis Sertoli cells undergo a cyclic transformation which is coextensive with the cycle of seminiferous epithelium. The Sertoli cells cycle may, in fact, be the most important coordinating factor in the spermatogenic cycle. It is but obvious that any diet that can influence the number of Sertoli cells, will in a way influence the diameter of the seminiferous tubule. The figures obtained and reported in this study are within the range earlier reported for the domestic fowl (De Reviers, 1971; Clermont, 1972 and kennelly, 1972). The diameter of spermatogonia A, though on the lower side, falls within the range 6-9 microns, reported by De Reviers, (1971). However, the reports of these findings agree with those of Nkanga and Egbunike (1990) of a typical humid condition.

CONCLUSIONS

Irrespective of the method of processing or diet type breeder cocks' reproductive potential was not in any way affected. Sweet potato can be used to improve on the portion of the germinal epithelium that enters the mitotic phase of spermatogenesis in the breeder cocks. It can equally be used for the production and selection of high quality sperm in breeder cocks for the subsequent use in Artificial Insemination (AI) in the production of healthy and good breeder cockerels as good genetic materials to be distributed to farms. Sweet potato is economical to substitute for maize in breeder cock diets production.

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